Population samples

A study of genetic diversity in coastal coho salmon was conducted on samples taken in 57 collections from 14 watersheds (Table 2; Fig. 1). Sites were chosen as representative of a wide geographic range beginning at the southern end of the Northern California/ Southern Oregon ESU and ending at the southern boundary of Central California Coast ESU. California Department of Fish and Game recognizes a split in the Central California Coast ESU at San Francisco Bay and has protected, under the California Endangered Species Act, samples south of San Francisco. All Russian River samples in our possession were included. Redwood Creek on the South Fork of the Eel River, the Noyo River, the Russian River, Olema Creek, Lagunitas Creek, and Scott Creek were sampled in different years, permitting study of temporal genetic variation. Across the 57 samples, 1745 individuals were available for genetic analysis (Table 2); LSGA95 (n=32), one LSGA96 (n=52), and the LSGY96 (n=10) were omitted from further analysis, owing to poor PCR results on the final tray.

Molecular methods

DNA from samples was extracted using the Puregene™ DNA isolation kit (Gentra System), a superior extraction procedure to Chelex 100 (BioRad) particularly when extracting tissue from degraded carcasses. DNA extractions were performed using 96-well trays. We made multiple attempts to extract and amplify samples that were initially unsuccessfully genotyped.

Individuals were genotyped for the seven microsatellites described in Table 1B, by first amplifying each microsatellite marker from genomic DNA via the polymerase chain reaction (PCR) and then separating the PCR products according to molecular size by polyacrylamide gel electrophoresis (PAGE). The forward PCR primer was labeled with a fluorescent phosphoamidite (HEX or fluorescein). PCR products were electrophoresed, 96 at a time, with allelic controls, on a 45.0 cm wide by 22.5 cm high 8% denaturing PAGE gel at 50 W for 150 min. DNA fragments were visualized on the FMBIO® fluorescent imaging system (Hitachi Software Engineering America Ltd). The relative sizes of individual bands were scored, using BIOIMAGE software. To control genotype scoring among trays, we co-electrophoresed eight individuals from each of the 20 trays in one set of gels. The data were double-checked for accuracy and independently verified by at least one other researcher. Individuals that did not produce repeatable genotypes and were difficult to score were not included in the analyses.

Statistical methods

*Population genetic parameters.* The raw genetic data comprise more than 1600 individual genotypes, such as these:

| ID | Ots103 | Ots-2 | i-Ots2 | Ots-3 | One-13 | P-53 | Oki-1 |
|---|---|---|---|---|---|---|---|
| KIGHA97051 | 236272 | 180182 | 217217 | 147153 | 000000 | 169183 | 088104 |
| KIGHA97053 | 220268 | 180180 | 205251 | 153153 | 201203 | 177181 | 088104 |
| KIGHA97046 | 248264 | 178184 | 205231 | 153157 | 215219 | 181181 | 096116 |
| KIGHA97048 | 220264 | 180182 | 205209 | 147153 | 000000 | 173181 | 096116 |
| KIGHA97019 | 276280 | 180182 | 221221 | 151153 | 211219 | 163169 | 096096 |

At the left is an individual identifier. Under each column headed by a marker name is a six-digit figure representing the two alleles scored for that marker in that individual. Each allele is represented by three-digits that correspond, roughly, to the size of the PCR product, in nucleotide base pairs. This number becomes a qualitative category, analogous, say, to alleles at a gene

controlling eye color in the fruit fly. For example, individual KIGHA97051 is heterozygous for the *236* and *272* alleles at the *Ots-103* locus. This same individual is homozygous for the *217* allele at the *iso-Ots-2* locus and is missing information at the *One-13* locus (represented as 000000, because a six-digit format is required for input into the GENETIX program described below). The fundamental quantitative data of interest are the frequencies of these allelic categories in populations. In this small example, the frequencies of the four alleles observed at the *Ots-2* locus in the five individuals shown above are: $f_{[178]}$=1/10 (0.1); $f_{[180]}$=5/10 (0.5); $f_{[182]}$=3/10 (0.3); and $f_{[184]}$=1/10 (0.1). The number of alleles is twice the number of individuals, and their frequencies sum to 1.0. From the H-W Principle, we would expect the frequency of *180180* homozygotes, for example, to be $(0.5)^2 = 0.25$ or 1 out 4; we observe 1 out of 5 such homozygotes in this small data set.

We tested the fit of genotypic proportions within populations to the Hardy-Weinberg equilibrium proportions, using GENEPOP, version 3.3 (available at ftp://ftp.cefe.cnrs-mop.fr/genepop/). Allele frequencies, observed and expected proportions of heterozygotes, and *F*-statistics ($F_{IS}$ and $F_{ST}$) were calculated, using the program GENETIX version 3.3 (available at http://www.univ-montp2.fr/~genetix/genetix.htm). The significance of pairwise linkage disequilibrium (LD), $F_{IS}$ and $F_{ST}$ tests was determined by performing 500 permutations of the data in GENETIX. For $F_{IS}$ and LD, these permutations were of alleles among individuals within a population; for $F_{ST}$, the permutations were of multi-loci genotypes among individuals from all populations. Significance was determined by the percentage of permutations yielding a value as large or larger than that observed, with the nominal 5% level being the threshold for rejecting the null hypothesis of $F$=0. Homogeneous sets of populations within sites were determined by testing the significance $F_{ST}$ among all of the samples from that site; if $F_{ST}$ is significant (*i.e.* 5% or fewer of the permutations yielded an estimate as large or larger as that observed), then the most divergent member of that group was removed and the $F_{ST}$ was re-tested. If significant, the next most divergent member was removed; this process was repeated until a set of homogenous populations with a non-significant amount of inter-population variance was obtained. The matrix of $F_{ST}$ among all pairs of populations is used to determine divergence of members from the rest of the group

Cavalli-Sforza and Edwards (1967) chord measures (CSE) were calculated using GENDIST in the program PHYLIP (Felsenstein 1993). Unweighted pair-group method with arithmetic mean (UPGMA) or average distance trees (Sneath and Sokal 1973) were calculated using NEIGHBOR in PHYLIP. Bootstrap results for assessing the frequency of occurrence, and thus significance, of each tree cluster were obtained using SEQBOOT and CONSENSE in PHYLIP with 1000 replicates. Trees were visualized using TREEVIEW (Page 1996). A Neighbor-Joining tree was constructed in PHYLIP for the individuals in the RRGVY98a sample, based on the allele-sharing distance metric in Msat2 (http:/hpgl.Stanford.edu/info@hpgl.Stanford.edu).

*Analysis of kinship and adjustments for family structure in juvenile samples.* We examined relatedness in juvenile samples and adjusted these samples for family structure, following the methods previously developed and published by us (Banks et al 2000). High levels of LD (more than two of 21 loci-pairs significant at 5% or lower) and significant departures from single-locus H-W equilibrium proportions indicated those samples in need of adjustment.

The odds of two individuals being full-siblings rather than unrelated were calculated, using the program KINSHIP 1.2 (Goodnight and Queller 1999). An appropriate baseline of allelic frequencies was derived from a pool of adults collected within the same ESU as the juvenile sample of interest. The LOD-score classification of a full-sib relationship between two individuals is conservative when applied to winter chinook test families, *i.e.* the test has low power, detecting a little more than half of true full-sibs, but suitable protection against Type-I error, classifying very few truly unrelated pairs as full-sibs. The number of loci, however, is critical to this test. We first deleted individuals missing more than three loci (though retaining the exceptionally polymorphic combination of *Ots-103*, *iso-Ots-2*, *Oki-1*), to avoid potentially spurious results in evaluating kinship.

The output of KINSHIP is a triangular half-matrix of relatedness coefficients, LOD scores, or test results from all possible pairwise kinship tests. This latter matrix becomes one input into SIBLINGS, a program written by Will Eichert to analyze the family structure of sampled individuals, following the methods of Banks et al (2000). The other inputs are allele frequencies for the appropriate baseline population and the genotypes of individuals in the juvenile sample. The significance of the relatedness test serves as an initial indicator of possible sibling groups. The SIBLINGS program examines potential sibling groups for violations of Mendelian rules of inheritance (*e.g.* more than 4 alleles at any locus or impossible combinations of genotypes). Any individuals not conforming to Mendelian rules are discarded from the group, though they become candidates for inclusion in other groups. The clustering and discard algorithm has difficulty parsing a sample that comprises many families with complex mixtures of full- and half-sibs, as happened in the RRGVY98a sample. Following Bentzen et al (2001), we partitioned this sample into smaller sets, using a Neighbor-Joining tree of allele sharing among individuals. Once smaller kinship groups were identified, the genotypes of the group's parents are reconstructed. The genotypes of possible parents must be able to produce the genotypes of all offspring (see Table 2, Banks et al 2002). Possible mating pairs are then scored and ranked. The score is a product of the sibling group's probability, under all relevant bi, tri, or tetranomial distributions, and the joint likelihood of the parental genotypes. After forming full-sib groups, SIBLINGS looks for families that have a common parent (half siblings). All individuals in each sibling group are then removed from the dataset, and replaced by their parents.

Adjustments of samples

Adult populations that departed significantly from random mating expectations were further examined for evidence of admixture, *i.e.* that deficiencies of heterozygotes in these samples might have resulted from Wahlund effect. Subdivision of a sample was only possible if independent information, such as size (fork length), collection date, or collection site, was available. In these cases, samples were subdivided, according to criteria specified in Table 2, and each subsample was re-tested for single and multi-loci random mating equilibria. $F_{ST}$ among subsamples was also calculated and tested for significance. Wahlund effect in the original sample would be evidenced by non-significant departures from H-W within subsamples but significant $F_{ST}$ among subsamples. Details on specific populations are given below.

Twenty-seven of the 57 collections comprised young of the year or smolts. Each of these juvenile samples required intensive effort to discriminate the contributions of population admixture (Wahlund effect) and family structure to its departure from random mating equilibrium. We first checked for admixture, if independent criteria permitted subdivision, as

described above for adult samples. We next applied the family adjustment procedure multiple times, altering both stringency of inclusion in kinship groups and minimum sib-group size, in a series of tests designed to find an optimum adjustment that minimized LD and the number of reconstructed parents, while maximizing the number of unrelated individuals. The large amount of family structure revealed in the RRGVY98a sample is detailed in the Results section; detailed accounts of adjustment procedures in each of the other juvenile samples follow. We also applied family adjustment to the Scott Creek adult samples from the Monterey Bay Trout and Salmon Project hatchery, which also showed substantial LD.

KIGHA

Eighty-one Klamath River, Del Norte County samples were collected from returning adults at the Iron Gate Hatchery (IGH) on 11/18, 11/24, or 12/18/1997. Biological data also included sex, fork length and marking type applied at time of release. We separated the 81 individuals into subgroups determined by the relevant and available biological information to determine whether heterogeneity existed among samples. There was no difference among samples based on collection date ($F_{ST}$ = 0.0038, $P$< 0.159). We separated individuals by mark type and fork length (FL). Returning adults had an adipose clip, a left maxillary clip, or were non-clipped. Adipose clipped fish are likely released from the Cole M. Rivers Hatchery on the Rogue River, OR, which in some cases is verified by the presence of recovered pit tags (personal communication, IGH staff). Non-clipped adults may be wild spawned or hatchery escapees, while left maxillary fish are returning IGH adults. We tested the frequency distribution of size by mark type to determine cut-off points for developing discrete sub-populations (Fig. 2). Thirteen left-clipped, and two non-clipped individuals, constitute a sub-population of precocious males or jacks (FL<56cm) (population KIGHAj, Table 2, where N=13), and likely represent an alternate year class. Large individuals (FL> 56cm) of all mark types generally follow a normal distribution (Fig. 2) and are initially considered as three separate sub-populations within the Klamath system. Sample sizes for large adipose-clipped, left-clipped, and non-clipped adults are 11, 36, and 19 respectively (populations KIGHAal, KIGHAll, and KIGHAnl, respectively). In tests for homogeneity among all four putative populations, only adipose-clipped and non-clipped could be combined $F_{ST}$=0.0044, $P$< 0.306 (Table 4). The number of loci-pairs showing significant linkage disequilibrium ($P$< 0.05) was high when considering the 81 samples represented a single population (8/21 loci-pairs).

TRHA

We analyzed a total of 94 adults collected at the Trinity River Hatchery (TRH) on November 12 or December 1, 1997. All adults were marked with a right-maxillary clip applied by TRH at the time of release. Fork lengths, date of collection, and sex were also provided for each individual. We partitioned the 94 individuals into smaller putative populations based on the available information to test for heterogeneity among samples. Samples collected on the two dates (11/12 and 12/1) were homogenous ($F_{ST}$ = 0.0024, $P$<0.253). We tested for heterogeneity among different size classes. Fork-length ranged from 36-74 cm, and there was a discrete separation between small males (36-44cm) and large (53-74cm) adults of both sexes. The jacks or small male category (sample TRHAs where N=17) and large category (TRHAl where N=77) were significantly heterogeneous ($F_{ST}$ = 0.0131, $P$<0.022).

LRS00

Little River, Humboldt County (LRS00) samples, were provided by Simpson Timber Co. from the Little River lower South Fork trap, spanning the dates April 3, to May 29, 2000. All samples were collected from out-migrating smolts. Data included sample collection date for individual samples. Nine loci-pairs out of 21 showed significant LD. We tested whether samples collected from different dates constituted a single homogeneous population. In cases where the number of out-migrating smolts collected on individual dates was insufficient, samples were binned to achieve adequate sample sizes. The 5 putative populations were grouped as follows: 4/3 (N=19), 4/4(N=38), 4/6(N=17), (4/20-5/6) (N=11), and (5/19-5/29) (N=11). The global $F_{ST}$ for these 5 populations was 0.0095 ($P$<0.014). The most divergent population (5/19-5/29) was removed and the $F_{ST}$ for the remaining 4 populations was 0.0036 ($P$<0.204). This indicates that the 85 individuals collected between 4/3 and 5/6 constitute a single homogenous (population LRS00-1, Table 2) that is not homogeneous with the 11 individuals collected between 5/19 and 5/29 (LRS00-2). After separating samples into two populations, eight out of 21 loci-pairs showed significant LD in population LRS00-1. We adjusted both populations for potential family structure with the program SIBLINGS. Two individuals were removed from LRS00-1 because they did not meet the minimum requirement of genotype values at four loci (or the acceptable combination of *Ots-103*, *iso-Ots-2*, and *Oki-1*). This reduced N= 85 to N=83 individuals. The SIBLINGS output pedigree for this population included 28 unrelated individuals, and 44 parents representing 23 Sibling groups (23 smolts were replaced by their hypothetical parents), totaling 72 individuals in the adjusted sample. The 11 LRS00-2 individuals were also corrected for family structure. Of the initial 11 individuals, five were unrelated and four parents, representing two sibling groups, replaced six. After adjustment, both sub-populations were subsequently homogenous ($F_{ST}$ = 0.0031 $P$<0.292), and the LD was reduced from 9/21 to 3/21 significant loci-pairs.

EREDS97

In 1997, out-migrating smolts were collected from Redwood Creek on the South Fork of the Eel River (population EREDS97). Of the 95 samples analyzed, 81 were collected on 4/26/97, and the remaining14, were collected on 4/30/97 (Eel River Restoration Salmon Project, Table 2). There was no available information, to separate the 95 samples into sub-populations. To correct for possible family structure, we analyzed 89 individuals that met the four (or three) locus criteria. The SIBLINGS pedigree included 52 unrelated individuals and 24 hypothetical parents comprising 13 different sibling groups. From an initial 2/21 significant loci-pairs, the adjustments for family structure reduced LD to 1/21 significant associations.

ESPRS99

In 1999, 34 out-migrating smolts were collected from the South Fork of Sproul Creek located on the South Fork of the Eel River (Eel River Salmon Restoration Project, Table 2). Accompanying data included date trapped and fork length. Fork length ranged from 68 to 110mm but showed a gap between 92mm and 96mm; thus, we formed two putative sub-populations of 68 to 94mm and 96 to 110mm. These populations were homogenous ($F_{ST}$ = 0.0066, $P$<0.20). Samples split into date trapped (4/5-4/22 and 5/10-6/4) were also homogenous ($F_{ST}$ = 0.015, $P$<0.072). Thirty-four individuals were tested for family associations using SIBLINGS. The program pedigree included 12 unrelated individuals, and 18 hypothetical parents comprising nine sibling groups

(Table 4). LD dropped from an initial 4/21 significant locus-pair associations, to 0/21 after adjustment for family structure.

MATS

Ninety-six Mattole River smolts were collected between 5/7/98 and 6/1/98 from the Mattole mainstem at river mile three by screw trap (Mattole salmon Group). Fork length and collection date were available. Three putative populations were constructed based on collection time 5/7-5/11, N=47, 5/12-5/16, N=28, and 5/19-6/1, N=21. The global $F_{ST}$ for three putative populations was 0.0077, $P<0.032$. Removal of 21, late-migrating individuals (5/19-6/1) resulted in a homogenous population (MATS-1) of early out-migrants ($F_{ST} = 0.0047$, $P<0.148$). LD was significant (5/21 loci-pairs), but lower than the initial 8/21 significant loci-pairs. The N=21 MATS-2 sub-population, exhibited an LD value of 1/21 significant loci-pairs. Before adjusting family structure in MATS-1, two individuals were dropped due to insufficient data. The SIBLINGS output pedigree included 27 unrelated individuals, 26 sibling groups, and 1 shared parent. However, the LD value remained high at 6/21 significant loci-pairs. To reduce LD, we selected only the 27 unrelated individuals and tested homogeneity with the MATS-2 sub-population ($F_{ST} = 0.0048$, $P<0.21$). This yielded a homogeneous population of 48 unrelated individuals (MATS).

PUDY98

Eighty Pudding Creek 1998, young of the year samples were acquired by two collectors, from different portions of the watershed on 9/23/98 and 10/27/98 (PUDYh N = 37, PUDYk N =43, Table 2). PUDYh samples were further divided into two groups based on collection location. Upper Pudding Creek samples (PUDYu N = 4) and one individual with insufficient data, were dropped from further analysis, making N = 32 for PUDYh. The global $F_{ST}$ for PUDYh and PUDYk was not significant at –0.0055 ($P<0.844$). However, after adjustment for family structure in SIBLINGS, LD was reduced only slightly to 9/21 from an initial 10/21 significant loci-pairs. Taking the two sub-populations separately, LD for PUDYh and PUDYk respectively, was 4/21 and 6/21 significant loci-pairs. To further reduce the LD, we removed all hypothetical parents from the separate SIBLING pedigrees and jointly analyzed only unrelated individuals. We specifically tested whether the 44 unrelated individuals from the two sub-populations were homogenous ($F_{ST} = -0.0062$, $P< 0.860$). The calculated LD for the adjusted population PUDY was 5/21 significant loci-pairs.

ALBY98

Eighteen young of the year samples were collected on 10/30/98 from Marsh Creek, a tributary of the Albion River (CDFG). Linkage disequilibrium was moderate (3/21 loci-pairs) for these 18 individuals. We corrected for family structure given that they were collected in-stream, from few pools, over a short distance. SIBLINGS detected two sibling groups, consisting of three individuals each. Six individuals were replaced with their hypothetical parents, which reduced the number of significantly associated loci-pairs from 3/21 to 1/21.

RRGV98a

Seventy young of the year samples were collected from Green Valley Creek, a tributary to the Russian River on 7/20/98. These samples were collected from a relatively small area and were not likely to be heterogeneous (see RRGV98b below). A substantial number (15/21) of loci-

pairs had significant LD. We were unsuccessful in reducing LD to less than 3/21 significant loci-pairs using the SIBLINGS program. We subdivided the 70 individuals into four putative sibling groups using a dendrogram based on allele-sharing (see Fig. 4). After identifying closely related individuals based on the number of shared alleles, we corrected for family structure using SIBLINGS. Of the four SIBLING pedigree outputs, the largest identified sibling group contained 25 individuals, which were replaced by their two hypothetical parents. SIBLINGS also identified three groups of two siblings, four groups of three siblings, and two groups of four siblings. In all cases, two hypothetical parents replaced each sibling group. The adjusted N of combined tests was 25 individuals. The LD for adjusted RRGV98a samples was 1/21 significant loci-pairs.

RRGV98b

Fifty eight young of the year samples were collected from Green Valley Creek, a tributary to the Russian River on 10/13/98. These samples were collected from the same location as population RRGV98a, which was collected three months earlier (Fawcett, Table 2). Individuals collected at the later date could have been the same individuals sampled on the earlier date, but we were unable to confirm this, because all individuals collected at the later date possessed intact caudal fins (a caudal fin genetic sample was taken on 7/20). We initially tested whether samples collected from different pools constituted a homogenous population. No heterogeneity was detected among RRGV98b samples, collected from different pool sites ($F_{ST}$ = -0.0083, $P$<0.816). In the unadjusted sample, 15/21 loci-pairs showed significant associations. To correct for family structure, we ran all individuals simultaneously through the program SIBLINGS (for comparison, see RRGV98a, MATERIALS). SIBLINGS created a total of 18 sibling groups, the two largest groups of which consisted of 15 and 8 full-siblings. There were also 11 sibling groups consisting of three individuals each, four sibling groups with four individuals each and one group with five (see Table 4). The adjusted N for this sample dropped from 58 to 39 including hypothetical parents. After adjustment for family structure, LD dropped from 15/21 significant loci-pairs to 7/21. We were unable to reduce LD further.

LSGAY98

In 1998, 21 young of the year samples were collected from San Geronimo Arroyo (BML spring class, Table 2). These samples displayed an LD value of 6/21 significant locus-pair associations. After adjustment for family structure using SIBLINGS, only 2/21 loci-pairs were significant. The adjusted population comprised 16 unrelated individuals and two hypothetical parents replacing a sibling group of three individuals.

OLEY98

Eighty-eight Olema 1998 young of the year samples were collected from four reaches spanning the area just downstream of Vendata to, and including, Blueline Creek. We initially tested whether samples collected from the five different reaches constituted a single homogenous population. The samples collected from Reach 5 were least like the downstream samples but were not significantly heterogenous ($F_{ST}$ = 0.0030, $P$< 0.20). Five out of 21 loci-pairs showed significant LD. We corrected family structure with SIBLINGS, which constructed a population of 53 unrelated individuals and 10 sibling groups. The largest sibling group included eight individuals, and there were five groups with four individuals and four groups consisting of three individuals each. After adjustment, 4/21 loci-pairs still showed LD.

WADY99

In 1999, fifty-nine young of the year samples were collected from three distinct areas of Waddell Creek. Twenty-three samples were collected at or around river mile (RM) 3.1, 19 samples were collected from RM 3.9, and 17 samples were collected from RM 4.7 (Smith, Table 2). The among-site global $F_{ST}$ was highly significant at 0.0370, $P<0.00$. Samples originating from RM 4.7 were heterogeneous to both RM 3.1 and 3.9 and were removed (WADY99up, Table 2). The $F_{ST}$ for the remaining 36 samples (RM3.1 and 3.9) was not significant at 0.005, $P<0.27$ (WADY99low, Table 2). The WADY99up population had LD of 2/21 loci-pairs, while the WADY99low population had 7/21 significant locus-pair associations. We corrected WADY99low for family structure. The adjusted WADY99low population consisted of 15 unrelated individuals, and eight sibling groups, the largest of which represented 7 full siblings. The adjustment reduced LD to 3/21 significant loci-pairs. After adjustment, the WADY99low population was still heterogeneous with WADY99up ($F_{ST} = 0.059$, $P<0.00$) and could not be combined.

SCA95A

Forty-one returning adult coho were collected at the hatchery on Scott Creek in 1995 (MBTSP, Table 2). Five out of 21 loci-pairs had significant LD, potentially caused by family structure. Adjustments for family adjustment proceeded, using SIBLINGS. Seventeen unrelated individuals and 11 sibgroups were formed, 10 were derived from sibling groups consisting of two individuals each, and one group had four siblings (Table 4). After adjustment, LD dropped to 1/21 significant loci-pairs.

SCA97A

Fifty-six adults returning to Scott Creek were trapped at the hatchery in 1997. Fifteen out of 21 loci-pairs had significant LD. Adjustments for family structure proceeded, using SIBLINGS which produced a pedigree comprising 16 unrelated individuals, four groups of sibling pairs, nine groups of three siblings, and 1 group of four siblings. The LD after adjustment was reduced to 4/21 significant loci-pairs.

SCA98A

Forty-two adults returning to Scott Creek were trapped at the hatchery in 1998. To reduce possible family structure in these samples (LD = 11/21 loci-pairs), we used SIBLINGS. Four samples were deleted from further analysis due to insufficient data. The SIBLINGS pedigree consisted of five unrelated individuals, and 18 hypothetical parents. The largest sibling group consisted of six individuals while the majority had three siblings (Table 4). Adjustments for family structure reduced the LD to 4/21 significant loci-pairs.

SCY99

Sixty young of the year coho were collected from various regions within the Santa Cruz Scott Creek watershed, in 1999 (Smith, Table 2). Ten individuals were collected from each of the following mainstem areas; RM 2.55, RM 3.55, RM 4.9, and tributaries, Big Creek, Mill Creek and Upper Fork totaling N=60. The global $F_{ST}$ for 60 samples separated by collection site was highly significant ($F_{ST} = 0.036$, $P< 0.00$). Upper Fork samples were the most heterogeneous and were removed. The $F_{ST}$ for the remaining sample sites was 0.0191 and still significant ($P<0.030$). The further removal of RM 4.9 samples (SCY99up) yielded a homogenous

population consisting of RM 2.55, RM 3.55, Big and Mill Creek samples (SCY99low, Table 2). Six out of twenty-one loci-pairs showed significant LD in the SCY99low population. The adjusted SCY99low population consisted of 12 unrelated individuals, and seven sibling groups, in most cases consisting of six to eight siblings per group (Table 4). After family adjustment, SCY99low was not homogenous with SCY99up, but the LD value was reduced to 3/21 significant pair associations.

**Results**

Genetic diversity within California Coastal Coho

Preliminary analyses of the genetic data suggested widespread departures from random mating expectations, as measured by tests of single-locus and multi-loci equilibria (2001 annual report). Although many of these deviations were observed in juvenile population samples, which are expected to deviate from random mating expectations, many samples of adults also appeared to depart from random mating equilibrium. First, we investigate the possibility that departures from random mating equilibrium within adult samples might have resulted from artificial admixture of fish from genetically different subpopulations.

The 1997 sample of 81 adults from the Klamath River Iron Gate Hatchery illustrates the Wahlund effect. $F_{IS}$ for this sample is 0.076, a value that is attained in none of the 500 permutations of the alleles among individuals (*i.e.* $P = 0.0$), and seven of 21 pairwise LD tests are significant at the 5% level. The distribution of fork lengths in the KIGHA97 sample shows a clear separation into jacks (males less than 56cm FL) and older adults (Fig. 2). The sample can also be subdivided by the presence and kind of mark (no mark, which could be either wild or

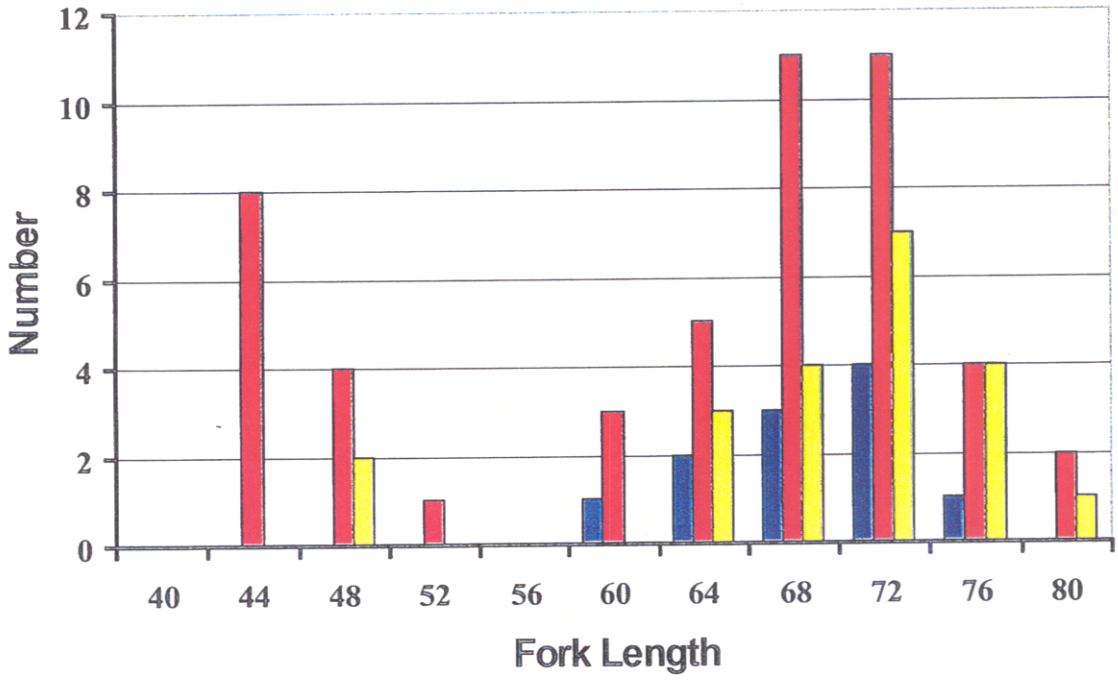


**Fig. 2.** Distribution, by fork length (cm) and mark, of adult coho salmon sampled from returns to the Iron Gate Hatchery, Klamath River, in 1997; blue bars are adipose fin clipped (Rogue River hatchery mark), red bars are left maxillary notched (the IGH mark), yellow bars are unmarked fish (wild or hatchery).

**Table 3.** Deviations from random mating genotypic proportions, by locus (*, P<0.05; **, P<0.01; ***, P<0.001) and over all loci ($F_{IS}$, $P$), and proportion of loci pairs showing linkage disequilibrium (LD) for 49 samples of coho salmon.

| Population | N | *Ots-103* | *Ots-2* | *Iso-Ots2* | *Ots-3* | *One-13* | *P-53* | *Oki-1* | $F_{IS}$ | $P$ | LD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| KIGHA97a | 11 | | | | | | | | 0.009 | 0.470 | 2/21 |
| KIGHA97j | 15 | | | * | | | | | 0.019 | 0.326 | 4/21 |
| KIGHA97ll | 36 | | * | ** | | * | | | 0.073 | 0.012 | 3/21 |
| KIGHA97nl | 19 | | | | | | | | 0.089 | 0.010 | 1/21 |
| TRHA97s | 17 | | | | | | | | 0.024 | 0.276 | 2/21 |
| TRHA97l | 77 | *** | | *** | | | | * | 0.062 | 0.000 | 4/21 |
| LRS00-1 | 85 | *** | | *** | *** | | ** | | 0.080 | 0.000 | 9/21 |
| LRS00-2 | 11 | | | ** | | | | | -0.014 | 0.668 | 6/21 |
| EHOLA97 | 16 | * | | | | | | | 0.064 | 0.132 | 3/21 |
| EREDS97 | 92 | | * | ** | | | | | 0.058 | 0.000 | 2/21 |
| EREDA98 | 22 | *** | | | | | | | 0.056 | 0.066 | 2/21 |
| ESPRS99 | 34 | | | | | | | | -0.020 | 0.720 | 4/21 |
| MATS98-1 | 73 | | | | | | * | * | 0.030 | 0.112 | 7/21 |
| MATS98-2 | 21 | | | | | * | | | 0.054 | 0.850 | 3/21 |
| PUDY98h | 32 | * | | | | | * | | 0.068 | 0.022 | 5/21 |
| PUDY98k | 43 | ** | * | | | | | | 0.070 | 0.012 | 9/21 |
| NOYA97 | 44 | | | | ** | | | * | 0.064 | 0.012 | 1/21 |
| NOYA99 | 43 | * | | * | | | | | 0.076 | 0.010 | 1/21 |
| ALBA98 | 22 | | | | | *** | | * | -0.012 | 0.642 | 6/21 |
| ALBY98 | 18 | | | | | | * | * | -0.023 | 0.706 | 3/21 |
| RRHA95 | 33 | ** | | | | | | | 0.057 | 0.018 | 3/21 |
| RRHA96 | 25 | | | | | | * | | -0.046 | 0.914 | 4/21 |
| RRHY97 | 7 | | | | | | * | | 0.120 | 0.060 | 5/21 |
| RRGVY97 | 8 | | NA | | | | | ** | -0.032 | 0.588 | 0/19 |
| RRGVY98a | 70 | *** | * | *** | | ** | * | *** | -0.048 | 0.968 | 15/21 |
| RRGVY98b | 58 | *** | * | *** | | * | | *** | 0.022 | 0.236 | 16/21 |
| RRGVY00 | 8 | | NA | | | | | * | -0.257 | 1.000 | 0/15 |
| LAGA96 | 8 | | | | | | | NA | -0.062 | 0.734 | 0/15 |
| LAGA97 | 7 | | | | | | | | 0.052 | 0.194 | 2/21 |
| LDGA96 | 9 | | | | | | | | 0.165 | 0.012 | 0/21 |
| LDGA97 | 10 | | | | | | | * | 0.086 | 0.106 | 2/21 |
| LSGA96 | 5 | | | | | | | | 0.138 | 0.096 | 0/21 |
| LSGA97 | 61 | | | | | | | | -0.014 | 0.718 | 4/21 |
| LSGY98 | 12 | | | | | | | | -0.062 | 0.870 | 1/21 |
| LSGAA96 | 25 | | | | | | | | 0.000 | 0.538 | 0/21 |
| LSGAA97 | 3 | | | | | | | | -0.042 | 0.672 | 0/21 |
| LSGAY98 | 21 | | | | | | * | * | 0.000 | 0.442 | 7/21 |
| OLEA96 | 70 | | | | *** | | | * | 0.105 | 0.006 | 6/21 |
| OLEA97 | 34 | | | | * | | * | * | -0.010 | 0.610 | 3/21 |
| OLEY98 | 88 | | * | ** | | | | | -0.010 | 0.560 | 5/21 |
| RWMA97 | 15 | ** | | ** | NA | | | | 0.113 | 0.090 | 0/18 |
| RWMY98 | 24 | * | | | | | | | -0.002 | 0.480 | 0/21 |
| WADY99low | 42 | ** | | ** | *** | | | | 0.011 | 0.356 | 7/21 |
| WADY99up | 17 | | | | | | * | | -0.085 | 0.900 | 2/21 |
| SCA95 | 41 | | | | | | | | -0.051 | 0.958 | 5/21 |
| SCA97 | 57 | * | | * | ** | | | | -0.047 | 0.966 | 15/21 |
| SCA98 | 38 | *** | | * | * | * | ** | * | 0.099 | 0.010 | 11/21 |
| SCY99low | 40 | | | | | | | | -0.028 | 0.780 | 7/21 |
| SCY99up | 20 | | | | | | | | -0.030 | 0.690 | 2/21 |